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UNITED STATES DISTRICT COURT

THE UNITED STATES DISTRICT COURT
DISTRICT OF NEW JERSEY

IN RE: Pet Food Products Liability Litigations	)	Civil Action No. 07-2867 (NLH) MDL DOCKET NO. 1850
	)	DONALD R. EARL'S RESPONSE TO MENU FOODS' REPLY RE:
	)	OBJECTION TO PET FOOD EVIDENCE DISPOSAL PLAN APPROVED ON DECEMBER 18, 2007

1. FACTUAL BACKGROUND AND ARGUMENT

On July 13, 2007, Earl filed a complaint against Menu Foods Income Fund and The Kroger Company, in an individual action in Washington state. Stellman Keehnel, of DLA Piper's Seattle, WA office, replaced Menu Foods' previously appointed counsel on October 1, 2007.

On January 14, 2008, Earl was contacted by Menu Foods' attorney of record by email, requesting Earl make a stipulation of no objection to this Court's December 18, 2007 order, which in part provided for the destruction of evidence Earl reasonably anticipated acquiring some part of for the purpose of testing on discovery. In subsequent discussions, Menu Foods threatened Earl with litigation for sanctions if he did not consent to the wholesale destruction of this body of evidence. As shown by the exhibit filed in Menu Foods' response, Menu Foods is in fact attempting to carry out this threat, with the sole justification being this Court's December 18, 2007.

That order was obtained in part, based on Menu Foods misrepresentation to this Court, on page 21 of its December 11, 2007 motion, which stated: "This Unorganized Inventory is of no discernable use to any party interested in future testing of the product."

Selected quotes from the UC Davis study on cyanuric acid and melamine toxicity in cats, (exhibit A):

"No effect on renal function was observed in cats fed with melamine or cyanuric acid alone."

"The reason for the presence of cyanuric acid is unknown, although it may also have been added intentionally..."

"No evidence of renal failure was observed after feeding melamine alone..."

"Exposure to cyanuric acid alone in the diet for a total of 10 days showed no evidence of renal failure..."

"Melamine or cyanuric acid administered alone, even at high doses of 181 mg/kg melamine and 243 mg/kg cyanuric acid, did not have any effect on renal function of cats..."

"This study has shown that a single oral exposure of cats to melamine and cyanurid acid at a concentration as low as 32 mg/kg each can result in acute renal failure."

In tests ordered by Earl, pet food manufactured by Menu Foods in early September of 2006 (exhibit B) tested positive for substantial quantities of cyanuric acid. This was a full two months before Menu Foods introduced its melamine gravy style pet food. This is an example of the type of evidence to be found in the so called "unorganized product" Menu Foods seeks to destroy. Based on the samples Earl had tested, the two cans of pet food contained approximately 28 mg. cyanuric acid.

"Unorganized" is a misnomer. Menu Foods has most carefully organized a large body of evidence into two categories: The evidence it would like plaintiffs to have, and the evidence it seeks to prevent plaintiffs from acquiring at all cost.

Furthermore, Menu Foods has stored this body of evidence in a manner which may only be construed as intentional spoilation. From warehouse photos of cardboard bins, it also appears effort was expended to randomize the evidence to the greatest degree possible in order to make identification and location of individual items nearly impossible.

Under the circumstances, it's difficult to appreciate the strategy of counsel for plaintiffs in this action. They have sought only to preserve evidence, which constitutes one half of a two part toxin, which alone cannot account for a proximate cause of damage. Leaving aside issues of other toxins known to have been found in the food, it's the lethal effect of feeding a combination of "organized product" and "unorganized product" to pets, which damaged plaintiffs in the actions related to melamine contamination. No amount of due diligence on the part of Earl could have anticipated parties to this action would abandon a body of evidence in which there should be a common interest in preserving. Earl will suffer imminent harm if his legally protected interest in the evidence is lost, as well as the prejudicial effect this Court's order is likely to have on litigation pending in Washington state due to the manner in which Menu Foods is seeking to leverage that order. As a favorable decision from this Court may redress the issue, Earl should be considered to have Title III standing to enter his objection, at the very least on a limited third party basis. Alternately, or additionally, provisions of Rule 24 should apply.

2. CONCLUSION

For the above reasons, Earl respectfully requests the Court recognize some form of limited standing, or right or permission to intervene, to the extent such intervention pertains to the preservation of some portion of the body of evidence known as "unorganized product".

Dated: February 12, 2008
Respectfully submitted by:

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EXHIBIT A

UC Davis study on melamine toxicity

JVDI Journal of Veterinary Diagnostic Investigation

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Full Scientific Reports

Assessment of melamine and cyanuric acid toxicity in cats

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Abstract

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The major pet food recall associated with acute renal failure in dogs and cats focused initially on melamine as the suspect toxicant. In the course of the investigation, cyanuric acid was identified in addition to melamine in the offending food. The purpose of this study was to characterize the toxicity potential of melamine, cyanuric acid, and a combination of melamine and cyanuric acid in cats. In this pilot study, melamine was added to the diet of 2 cats at 0.5% and 1%, respectively. Cyanuric acid was added to the diet of 1 cat at increasing doses of 0.2%, 0.5%, and 1% over the course of 10 days. Melamine and cyanuric acid were administered together at 0%, 0.2%, 0.5%, and 1% to 1 cat per dose group. No effect on renal function was observed in cats fed with melamine or cyanuric acid alone. Cats dosed with a combination were euthanized at 48 hours after dosing because of acute renal failure. Urine and touch impressions of kidneys from all cats dosed with the combination revealed the presence of fan-shaped, birefringent crystals. Histopathologic findings were limited to the kidneys and included crystals primarily within tubules of the distal nephron, severe renal interstitial edema, and hemorrhage at the corticomedullary junction. The kidneys contained estimated melamine concentrations of 496 to 734 mg/kg wet weight and estimated cyanuric acid concentrations of 487 to 690 mg/kg wet weight. The results demonstrate that the combination of melamine and cyanuric acid is responsible for acute renal failure in cats.

Key Words: Cats • cyanuric acid • feline • kidney • melamine • nephropathy • pet food recall • toxicosis

Introduction

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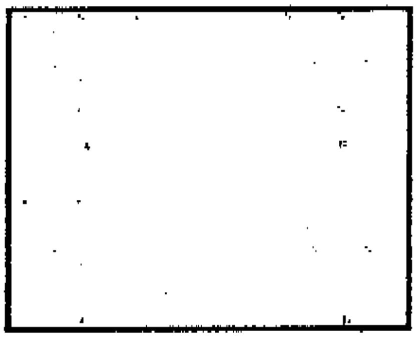
In March 2007, numerous cases of acute renal failure in dogs and cats were associated with the ingestion of a variety of dog and cat pet food products (http://www.fda.gov/oc/opacom/hottopics/petfood.html).3 At first, the suspected contaminant was identified as melamine. Melamine is a chemical used primarily for the production of melamine resins,2,19,20 but it has also been marketed as a fertilizer because of its high nitrogen content.18 In the case of the pet food, it has been speculated that melamine was added intentionally to raise the apparent protein content of the food, as the protein concentration is measured by analysis of total nitrogen content (http://www.nytimes.com/2007/04/30/business/worldbusiness/30food.htmlex1189742400en9565d7aa6a8e In Asia in 2004, a major outbreak of renal failure occurred in dogs, which showed identical clinical, histologic, and toxicologic findings to the outbreak in North America in 2007.3

Melamine (2,4,6-triamino-1,3,5-triazine; Fig. 1A) is considered to be relatively nontoxic, but specific data on toxicity, especially in cats, is lacking. In a 4-week study in dogs, oral administration of 125 mg/melamine/kg body weight had a diuretic effect, but no other effects were observed.13 In the course of the pet food recall investigation, cyanuric acid was also identified in the pet food as a co-contaminant (McPheron T: Melamine and cyanuric acid interaction may play part in illness from recalled pet food, AVMA Press Release, 5/1/2007). The reason for the presence of cyanuric acid is unknown, although it may also have been added intentionally or perhaps was a by-product of melamine synthesis (New York Times, Another chemical emerges in pet food case, 05/09/2007). Cyanuric acid (s-triazine-2,4,6-triol; Fig. 1D) is structurally related to melamine and used as a stabilizer in outdoor swimming pools and hot tubs to minimize the decomposition of hypochlorous acid by light.9 However, only limited data on the toxicity of cyanuric acid to mammals exist. Sodium cyanurate fed subchronically to rats and mice at up to 700 mg/kg and 2,200 mg/kg, respectively, caused bladder calculi and some associated bladder epithelial changes but no other adverse effects.10

Figure 1 Structures of melamine and analogs. A, melamine; B, ammeline; C, ammelide; D, cyanuric acid.



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Although melamine and cyanuric acid are relatively nontoxic individually, no data could be found that have determined the potential toxicity of melamine and cyanuric acid in combination. The purpose of this study was to assess the toxicity of melamine and cyanuric acid alone and in combination when fed to cats and to describe any clinical pathologic changes or gross or histologic lesions, thereby providing crucial information for evaluating the significance of the contaminants in the recalled pet food.

Material and Methods

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Animals
The protocol for this study was approved by the Animal Care and Use Committee of the University of California at Davis, and studies were conducted in compliance with the Animal Welfare Act, U.S. Public Health Service Policy on the Humane Care and Use of Laboratory Animals, and the Guide for Care and Use of Agriculture Animals in Agriculture Research and Teaching. The study was carried out in 3 parts using a total of 4 female adult cats with body weights of 3.5, 3.5, 4.7, and 4.9 kg, respectively. Cats were housed individually in mesh stainless steel cages in the same air-conditioned room in compliance with established standards and were provided access to water ad libitum. Prior to the feeding trial, cats were acclimated for 1 week during which they received commercially available wet cat food once daily.

Experimental Design
Melamine[a] and cyanuric acid[b] were commercially obtained and added to the wet food diet at concentrations of 0.2%, 0.5%, and 1%, separately or combined. Wet food used in this study was analyzed and found to be free of either contaminant at or above 10 mg/kg. Cats were fed once daily in the afternoon. Food consumption was calculated based on the amount of food offered and the amount of food remaining prior to the next feeding. Based on the concentration of melamine and/or cyanuric acid in the

food and the amount of food consumed, the doses of melamine and cyanuric acid in mg/kg body weight were determined. In part 1 of the study, 2 cats weighing 3.5 kg (cat 4) and 4.7 kg (cat 3) received melamine in their diet at 0.5% and 1%, respectively. Dosing of melamine was done once daily for 11 days. Routine serum chemistry and blood hematology parameters were obtained prior to administration of melamine and on days 3, 5, and 8 of the study. Since no adverse effects were noted in either cat, they were subsequently used in part 2 of the study, which began 10 days after completion of part 1. In part 2, 3 cats weighing 4.9 kg, 3.5 kg, and 4.7 kg received a combination of 0.2% melamine and 0.2% cyanuric acid (cat 2), 0.5% melamine and 0.5% cyanuric acid (cat 4), and 1% melamine and 1% cyanuric acid (cat 3), respectively. The combination was given once daily for 2 days. Serum chemistries were obtained at 36 hr after the first dose, and all 3 cats were euthanized using sodium pentobarbital at 48 hr after initial dosing. Criteria for euthanasia, as defined in the protocol, were a 2-fold or greater increase in serum urea nitrogen and creatinine, and partial to complete anorexia. A fourth cat (cat 1) served as a negative control and received the same food sample without the addition of cyanuric acid and melamine. In part 3 of the study, the control cat from part 2, weighing 3.5 kg (cat 1), received cyanuric acid daily at a concentration of 0.2% (4 days), 0.5% (3 days), and 1% (3 days). One day after completion of the cyanuric acid feeding trial, serum was obtained for clinical pathology, and the cat was euthanized using sodium pentobarbital.

Pathology

Necropsies including histology were performed on cats dosed with the combination of cyanuric acid and melamine (cats 2, 3, and 4) and the cat dosed with cyanuric acid alone (cat 1). Portions of brain, bladder, kidney, ureter, urethra, liver, lung, spleen, heart, pancreas, stomach, duodenum, jejunum, colon, thyroid, parathyroid, adrenal gland, thymus, lymph nodes (multiple, including mesenteric, retropharyngeal), bone marrow, and skeletal muscle were collected from all cats and immersed in 10% buffered neutral formalin. In addition to placing all tissues in 10% neutral formalin, kidney, brain, and lung tissues were also placed in 95% ethanol and in Bouin's fixative. Tissues were embedded in paraffin, sectioned at 5 μm, and mounted on positive-charged glass slides.[c] Tissue sections were stained with hematoxylin and eosin (HE). In addition, touch impression smears were made of the kidneys, and urine was collected for wet-mount examination.

Toxicology

Food and kidney samples were analyzed for melamine and its analogs, cyanuric acid, ammeline,[d] and ammelide[e] (Fig. 1) using liquid chromatography/mass spectrometry (LC/MS).[f,g] Melamine cyanurate, melamine, and its analogs were extracted from kidney tissue by homogenization in acetonitrile/water/diethylamine. Cyanurate is the anion of cyanuric acid and can form salts with cations. The solvent mixture dissociates melamine cyanurate into free melamine and cyanuric acid, which are subsequently analyzed by LC/MS. After dilution and sonication to remove proteins, an aliquot of the extract was evaporated to dryness under nitrogen. The extract was reconstituted, $^{15}N_3$-melamine was added as an internal standard, and the extract was analyzed by LC/MS using atmospheric pressure chemical ionization. Analysis was performed using selective reaction monitoring for 2 precursor-product ion transitions for each analyte, with one ion serving as the quantitation ion and the second ion as the qualifier ion. Cyanuric acid, ammeline, and ammelide were analyzed in negative ion mode, while melamine was analyzed in positive ion mode. Analytes were identified by retention time and their characteristic product ion ratios (Table 1). All 4 compounds were quantified using the $^{15}N_3$-melamine internal standard. The instrument was calibrated by daily analysis of the 5-point calibration curves of the analytes in solvent ranging in concentration from 50 ng/mL to 1,000 ng/mL. Method performance was evaluated by analysis of control tissue samples fortified with crystalline melamine cyanurate[h] as well as separate control samples fortified with melamine and its analogs. These analyses demonstrated that 75% to 95% of the melamine cyanurate fortified into tissue was recovered as melamine and cyanuric acid. This LC/MS method allowed detection of melamine, cyanuric acid, ammeline, and ammelide at concentrations of 5 mg/kg. All melamine and cyanuric acid concentrations in food and kidneys are expressed on a wet weight basis.

View this table: [in this window] [in a new window] Table 1 LC/MS parameters: analyte ions monitored and typical ion ratios and retention times.

Results

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No evidence of renal failure was observed after feeding melamine alone at 0.5% or 1% for 11 days. The daily dose of melamine was 181 mg/kg body weight for cat 3 that finished her food every day (1% melamine in diet). Cat 4 received melamine at a daily dose of 44, 60, 61, 121, 110, 121, 121, 121, 121, 119, and 95 mg/kg body weight (0.5% melamine in diet). Exposure to cyanuric acid alone in the diet for a total of 10 days showed no evidence of renal failure as measured by serum creatinine and urea nitrogen. The daily dose of cyanuric acid of cat 1 was calculated to be 49 mg/kg body weight daily for 4 days (0.2%), 121 mg/kg body weight daily for 3 days (0.5%), and 243 mg/kg daily for 3 days (1%). The apparent lack of toxicity of melamine and cyanuric acid when given individually was in sharp contrast to the toxicity of the combination. Approximately 12 hours after dosing with melamine and cyanuric acid combined, the 3 dosed cats developed slight depression, vomiting, and anorexia. The 3 cats had received the following doses on day 1: 32 mg/kg body weight of melamine and 32 mg/kg body weight of cyanuric acid (0.2%, cat 2); 121 mg/kg body weight of melamine and 121 mg/kg body weight of cyanuric acid (0.5%, cat 4); and 181 mg/kg body weight of melamine and 181 mg/kg body weight of cyanuric acid (1%, cat 3); respectively. The cats did not eat, or only partially ate, the second spiked food sample offered on day 2. The estimated ingested doses following the second feeding were as follows: 2 mg/kg body weight of melamine and 2 mg/kg body weight of cyanuric acid for cat 2 (0.2%); 10 mg/kg body weight of melamine and 10 mg/kg body weight of cyanuric acid for cat 4 (0.5%); and 54 mg/kg body weight of melamine and

54 mg/kg body weight of cyanuric acid for cat 3 (1%). Renal function was significantly impaired in all 3 cats when assessed at 36 hours after the initial melamine and cyanuric acid exposure (Table 2).

View this table: Table 2 Serum chemistry parameters at 36 hours postexposure in cats dosed with a combination of melamine (M) and cyanuric acid (CA).
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Among the 3 cats dosed with combinations of melamine/cyanuric acid, gross findings were similar, and atypical findings were limited to the kidneys (3/3 cats). Amorphous, rounded, and fan-shaped crystals were detected by wet mount of urine (Fig. 2A). Individual kidneys ranged between 0.85% and 1.1% of body weight. The kidneys bulged slightly within the retroperitoneal space. On section, the parenchymal surface and subcapsular regions were wet and oozed pale pink, slightly opaque fluid. The corticomedullary junctions were obscured by a broad, dark-red band that extended by fine, irregular tendrils into the cortex and the outer stripe of the medulla (Fig. 2C). By magnified inspection (dissecting scope or zoom lens of a camera), fine dots to 0.1-cm long threads of an opaque, variably white to yellow substance coursed from the papilla of the medullas into the obscured corticomedullary junctions (Fig. 2D). These were in parallel with the medullary rays and were most concentrated at the renal crest. Touch impression smears were made of the cut surface of the kidneys in each case. Crystals were tightly associated with tubules and were fan-shaped to amorphous, refractile, and birefringent (Fig. 2B). In cat 1, dosed with cyanuric acid alone, no gross or histologic abnormalities were present.

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Figure 2 Renal lesions present in cats dosed with melamine and cyanuric acid. **A**, wet mount, urine, cat 4; crystals in urine taken at the time of necropsy; crystals were clear and of variable sizes with rounded edges; Bar = 25 µm. **B**, touch impression, kidney, cat 2; crystals associated with intact, whole tubules were fan-shaped and iridescent; Dif-Quik stained; Bar = 25 µm. **C**, kidney, cat 2; the corticomedullary junction is obscured by a broad, dark-red band of hemorrhage; Bar = 0.3 cm. **D**, kidney medulla, cat 2; pale-yellow crystals visible within collecting tubules; Bar = 0.1 cm. **E**, kidney, cortex, cat 3; long, fan-shaped crystals within a tubule; tubular epithelium is attenuated and inflammatory cells are present within the adjacent interstitium; hematoxylin and eosin (HE) stain; Bar = 25 µm. **F**, kidney, renal papilla, cat 4; multiple crystals distending collecting tubules; HE stain; Bar = 200 µm. **G**, kidney, renal pelvis, cat 3; piling up of urothelial cells of the renal pelvis; individual cells are swollen and contain 1 or more cytoplasmic vacuoles; HE stain; Bar = 25 µm. **H**, kidney, corticomedullary junction, cat 3; interstitial hemorrhage and edema; tubular epithelial cells are variably necrotic; HE stain; Bar = 25 µm.

Histologic lesions of animals dosed with the combination of melamine and cyanuric acid were limited to the kidneys. Lesions within the kidneys were similar but with some variation in severity. In general, lesions correlated with dosage, with the highest dosage producing the most severe lesions. Crystals were present within the lumina of collecting ducts and within distal tubules (Fig. 2E, 2F). Crystals were translucent, pale yellow to clear, and in morphology varied from fan-shaped to starburst prisms to globular, sometimes filling segments of the tubule (Fig. 2E). All crystals displayed multicolored birefringence to cross-polarized light. The number of crystals in a given region of the kidney varied with kidney architecture and dose; in the most severe case (cat 4; 1% melamine and 1% cyanuric acid) an average of 50 crystals per 1-cm-diameter region of a 5-µm section were scattered within the cortex. Crystals were densely present in collecting tubules that occupied the renal crest (Fig. 2F). In cat 2 (0.2% melamine and 0.2% cyanuric acid), 20 crystals were present in the same diameter area of the cortex, and no crystals were visualized in the medullary region in sections examined. The number of crystals in each case was approximately equal in tissues fixed for 24 hours in either 95% alcohol or 10% buffered formalin. Crystals were not visualized in kidney tissues fixed for 24 hours in Bouin's fixative.

In general, the interstitium was edematous, and tubular architecture and epithelium throughout the nephron were affected. Within the cortex, proximal tubular epithelial cells were swollen and distended with fine to coalescing vacuoles. Both cortical tubules of the distal nephron and Bowman's space were often dilated. At the corticomedullary junction, in the region of the distal straight tubules, the interstitium was expanded by both edema and hemorrhage (Fig. 2H). Tubular epithelial cells in this region, both adjacent to and remote from the presence of crystals, had finely granular eosinophilic cytoplasm. Scattered cells or small groups of cells were shrunken with hypereosinophilia and pyknotic nuclei (necrotic). Rarely, degeneration was accompanied by clusters of neutrophils and lymphocytes. In cats receiving the combinations of 0.5% (cat 4) and 1% (cat 3), eosinophilic granular to hyaline protein casts were present within lumina. Also in these 2 animals, vascular endothelium was reactive with scattered segments of the vessel walls obscured by small clusters of inflammatory cells and fibrin. Arcuate and interlobar vessels and the vasa recta were congested. In all cases, but most marked in the animal receiving the highest dose (1%), individual urothelial cells of the renal pelvis were swollen, protruded into the lumen, and often contained 1 or more vacuoles (Fig. 2G).

Toxicologic analyses for detection of melamine, ammeline, ammelide, and cyanuric acid were performed on kidneys of all 4 cats. Melamine and cyanuric acid were detected in all 3 animals from study part 2 (Table 3). None of the kidneys contained ammeline or ammelide. In addition, the kidney of the cat receiving cyanuric acid alone (part 3 of study) contained 22 µg/g of cyanuric acid, but no melamine, ammeline, or ammelide.

View this table: [in this window] [in a new window] Table 3 Dose of melamine (M) and cyanuric acid (CA) in cats with M/CA-associated acute renal failure and kidney M and CA concentrations.

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Melamine or cyanuric acid administered alone, even at high doses of 181 mg/kg melamine and 243 mg/kg cyanuric acid, did not have any effect on renal function of cats based upon normal serum creatinine and urea nitrogen concentrations. The relatively nontoxic nature of melamine and cyanuric acid noted in this study is consistent with the limited toxicologic information that has been published for these compounds. The acute toxicity of melamine in rodents is reported with oral lethal doses 50 (LD50s) of 3,100 mg/kg (male rats) and 3,900 mg/kg (male mice).16 At doses of 150 mg/kg melamine or higher given to male rats for 90 days, there was a treatment-related effect of formation of bladder stones, which was indirectly responsible for urinary bladder neoplasia.16 No bladder stones were observed in female rats at dietary concentrations 10 times above the concentration that resulted in bladder stone formation in male rats. In 1 study in dogs, oral administration of 125 mg/melamine/kg body weight had a diuretic effect, but no other effects were observed.13 In sheep, a single dose of 2.17 g of melamine/kg body weight via rumen fistula resulted in increases in serum urea nitrogen. When melamine was administered daily at 10 g/animal, 2 sheep developed anorexia and anuria, and died at approximately 16 days and 31 days, respectively.7 No ill effects were observed in the 2 sheep until the sudden onset of clinical signs 3 days before death. A third dosed sheep remained unaffected over a dosing period of 39 days.

The toxicity of derivatives of cyanuric acid has been evaluated in rats and mice. Subchronic oral administration to rats and mice of sodium cyanurate at 700 mg/kg and 2,200 mg/kg, respectively, caused bladder calculi and associated bladder epithelial hyperplasia, but no other adverse effects.10 Of 3 dogs given 8% monosodium cyanurate in their daily diet, 2 dogs died after 16 months and 21 months, respectively, while the third dog was euthanized at the end of the 2-year study.11 Histologic lesions in all 3 dogs included kidney fibrosis and focal dilation. In contrast, dietary monosodium cyanurate given to dogs for 6 months at a concentration of 0.8% in their diet did not result in ill effects or renal lesions. Because of the low water solubility of sodium cyanurate, it was concluded that the compound precipitates at very high concentrations in urine and, thus, can lead to renal dysfunction.

To the authors' knowledge, there are no reports of toxicity studies examining the combined effects of melamine and cyanuric acid in any animal species. This study demonstrates that a combination of melamine and cyanuric acid administered orally at levels similar to those detected in contaminated pet food causes acute renal failure in cats. Melamine levels in pet food samples submitted to and analyzed by the California Animal Health and Food Safety (CAHFS) toxicology laboratory ranged from 10 mg/kg (0.001%) to 3,200 mg/kg (0.32%). While cyanuric acid was detected at levels greater than 10 mg/kg in many of the pet food samples submitted to this laboratory, further quantitation was not performed at the time. In the present study, cats receiving melamine and cyanuric acid at a minimum dietary concentration of 2,000 mg/kg each (0.2%) developed clinical signs of vomiting, weakness, and anorexia within 24 hours of a single exposure and had significantly elevated serum urea nitrogen and creatinine at 36 hours postexposure. The different concentrations of melamine and cyanuric acid in the diet did not appear to result in dose-dependent effects either clinically or clinicopathologically. Acute renal failure was observed at the lowest administered doses of melamine and cyanuric acid of 32 mg/kg body weight.

In contrast to the clinical and clinicopathologic findings, the extent of lesions generally correlated with the dose and included tubular damage, interstitial edema, and crystals. The lesions observed in this study were compatible with the pathologic findings of the acute phase of melamine-associated renal failure described in dogs and cats of the recent outbreak.[3] In addition, the morphology and location of crystals in cats dosed with a combination of melamine and cyanuric acid are the same as reported in dogs and cats developing renal failure as a result of contaminated pet food.[3] Azotemia was most likely associated with the tubular damage since visible crystals were only segmentally or multifocally occlusive. In addition, prerenal azotemia may have contributed to the increases in blood urea nitrogen and creatinine. The increases in anion gap observed in all 3 cats dosed with the combination of melamine and cyanuric acid may be a result of cyanuric acid or melamine metabolites, or other not-yet-determined unmeasured anions.

The mechanism associated with renal damage is unknown, but may, at least in part, be due to the intratubular precipitation of crystals in the kidney. The formation of crystals in the urinary tract is a complex process. Acute renal failure in humans associated with uric acid precipitation following tumor lysis is well described.[4,8,12,14] In addition, several commonly prescribed drugs such as acyclovir, sulfonamides, methotrexate, indinavir, and triamterene can lead to intratubular crystal formation, urinary obstruction, and acute renal insufficiency.[17] Dalmatian dogs are known to have heritable defects in uric acid metabolism with subsequent high levels of uric acid excretion. These dogs have been used to study the role of inhibitors in the pathogenesis of crystal formation and recent results indicate the inhibitory effect of certain macromolecules, such as glycosaminoglycans, on crystal formation.[6] It has also been hypothesized that renal damage occurs secondary to an inflammatory response caused by the crystals.[12] Alternatively, intrarenal urinary blockage might also contribute to renal tubular damage; although in affected cats, as mentioned, significant tubular occlusion was not noted. Based on the findings from this study, the exact mechanism for acute renal failure cannot be determined, but acute intrarenal obstruction appears to play a significant role. However, it is possible that a combination of factors, such as inflammation, obstruction by proteinaceous material secondary to casts, cell death, and obstruction by the crystals themselves is needed for the development of acute nephropathy.

Mixing melamine and cyanuric acid together in cat urine resulted in rapid crystal formation (Dr. Brent Hoff, personal communication, April 2007). The crystals were determined to be identical to those detected in urine from affected animals. Of interest, melamine and cyanuric acid rapidly combine into a lattice structure via hydrogen bonds.[21] The formation of insoluble complexes between cyanuric acid and melamine appears to be pH dependent. This reaction is the basis for gravimetric and turbidimetric methods to determine the levels of cyanuric acid and chlorinated isocyanurates in swimming pools.5 Urinary crystals were reported in a 49-kg sheep that died following a single 100-g intraruminal dose of melamine.7 Two sheep that were given total doses of 10 g per day for 16 and 31 days also developed crystalluria. Unfortunately, the nature of the crystals was not described. No crystalluria was noted in the 2 cats initially dosed with melamine at up to 181 mg/kg for 11 days. Based on these findings, it is postulated that the acid urine of cats led to the formation of insoluble melamine-cyanurate crystals. The precipitation of these crystals contributed to obstructive renal failure. Renal impairment from direct renal tubule toxicity of melamine-cyanurate may have also occurred. Because both dogs and cats have acidic urine, it is likely that the formation of insoluble melamine-cyanurate complexes was also responsible for the sickness and death of dogs. Although both dogs and cats developed acute renal failure following ingestion of contaminated food, anecdotal evidence suggests that more cases occurred in cats. The relative sensitivity of dogs and cats is unknown, but it is possible that species differences in renal physiology might have contributed to variable crystal precipitation within renal tubules. Individual risk factors might also have played a role, since the risk for drug-induced crystal formation is dependent on a variety of underlying conditions that lead to renal hypoperfusion.17 At the present time, there is insufficient epidemiologic information to assess coexisting risk factors that might have contributed to acute renal failure.

There are limited data with regard to melamine or cyanuric acid kinetics or metabolism following ingestion. In rats, 90% of a dose of radio-labeled melamine was eliminated unchanged via the kidneys within 24 hours.15 The plasma elimination phase half-life was determined to be 2.7 hours with a urinary excretion half-life of 3 hours. No residual radioactivity was noted in tissue other than the bladder at 24 hours or longer after dosing. In humans, more than 98% of an orally administered cyanuric acid dose is excreted unchanged in urine within 24 hours with a urinary excretion half-life of 3 hours.1 Thus, melamine and cyanuric acid do not appear to be subject to metabolism, which is not unexpected considering the triazine ring structure present in both compounds.10,15 This is supported by the findings of this study, because kidney tissues of cats dosed with a combination of melamine and cyanuric acid did not contain ammeline or ammelide. Thus, metabolism of melamine to ammeline, ammelide, and cyanuric acid as identified in microbes,18 does not appear to be present in cats. However, if melamine and cyanuric acid are administered in combination, formation of melamine-cyanurate crystals occurs. In the present study, melamine-cyanurate crystals were dissolved, and the individual compounds subsequently analyzed as melamine and cyanuric acid. It is not possible to estimate the contributions of melamine-cyanurate to the measured individual melamine and cyanuric acid concentrations. The measured concentrations of both compounds are similar to the concentrations identified in case submissions to the CAHFS toxicology laboratory of animals suspected of having died from melamine-cyanurate–associated acute renal failure. This confirmation of exposure is important in the diagnosis of melamine-cyanurate toxicosis, especially in the context of the major pet food recall.

This study has shown that a single oral exposure of cats to melamine and cyanuric acid at a concentration as low as 32 mg/kg each can result in acute renal failure. The clinical, clinicopathologic, toxicologic, and necropsy data confirmed acute renal failure. Detectable concentrations of melamine and cyanuric acid in kidneys also provided valuable diagnostic data. In cases of acute renal failure in cats in which other toxicologic etiologies can be eliminated, acute melamine and cyanuric acid toxicosis should be considered, especially if there is a history of a cluster of cases with similar dietary intake. In order to provide more extensive data that could be used for the risk assessment of contaminated food, future research studies are needed to determine the lowest dose of melamine and cyanuric acid that can cause renal failure.

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From the California Animal Health and Food Safety Laboratory System, Toxicology Laboratory (Puschner, Poppenga, Filigenzi), and the Department of Pathobiology, Microbiology and Immunology (Pesavento, Lowenstine), School of Veterinary Medicine, University of California, Davis, CA.

[a] Melamine, Sigma-Alrich, St. Louis, MO.

[b] Cyanuric acid, TCI America, Portland, OR.

[c] Superfrost/plus, Fischer Scientific, Pittsburgh, PA.

[d] Ammeline, generous gift from Dr. Fred Fricke, U.S. Food and Drug Administration, Forensic Chemistry Center, Cincinnati, OH.

[e] Ammelide, generous gift from Dr. Fred Fricke, U.S. Food and Drug Administration, Forensic Chemistry Center, Cincinnati, OH.

[f] Microm Biosystems high performance liquid chromatography, Microm BioResources, Auburn, CA.

[g] Applied Biosystems 4000 Qtrap, Concord, Canada.

h. Melamine-cyanurate, Sigma-Aldrich, St. Louis, MO.

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EXHIBIT B

Cyanuric acid positive lab report

ExperTox Inc. Analytical Laboratory
Accutrace Testing

Procedure: Verification Test (confirm for specimen)
Procedure Results: Detected
Job Location:

Specimen: Other
Date Recieved: 05/29/07
Date Reported: 06/08/07

Reason: Other
LName: Earl
FName: Don

ID: Mixed Grill
Accession#: 071490046

Drug	Results			
Verification Test	Detected	Cyanuric Acid 90.72 ug/g		

Details:
Details: Mixed Grill Pet Pride Cat Food composite from separate cans w/ the following lot numbers:
1.) Best By Sept 07 09 13:49
Made in USA FMG1S4
2.) Best By Sept 07 09 13:49
Made in USA FMG1S4

(This sample was sealed and in the original packaging)

Testing of the above product does not warrant that all products of this particular brand, lot or batch will produce the same results nor indicate that the entire product line is safe or not safe for consumption. This analysis is based on a small representative sample of the product provided and does not constitute full analysis of the entire product line, lot or batch. The results provided analysis from the sample submitted utilizing GC/MS.